Micro-Autoradiography Study of L-Methionine Distribution in Tumor Tissue: Effects of Radiotherapy

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Introduction

L-[methyl-^{11}C]methionine (^{11}C-Met) has been used for tumor imaging with positron emission tomography(PET). We have previously studied the characteristics of ^{11}C-Met uptake response to treatment using an *in vivo* rat tumor model. Tumor uptake of ^{11}C-Met, as a whole tumor showed a rapid decrease after radio-therapy, preceding spread of necrosis and tumor shrinkage[1]. In the present study, we examined micro-autoradiographically the distribution of methionine labeled with carbon-14 at the same position of carbon-11 using a rat tumor model following irradiation. This study was designed to elucidate the in vivo response of tumor cell uptake of Met following irradiation at the cellular level.

Materials and methods

The experimental protocol was approved by the Laboratory Animal Care and Use Committee of Tohoku University.

A 0.1 ml suspension of 7×10^6 AH109 A cells was subcutaneously inoculated in the thigh of nine young male Donryu rats weighing 170-190 g. Eight days later, 6 rats were anesthetized and tumors were exposed to a single dose of 20 Gy ^{60}Co irradiation as described previously[1]. The other three rats were served as the control. Three of each, one and two days post-irradiation and the control rats were given i. v. injection of 20 μCi(740 kBq) of L-[methyl-^{14}C]methionine (Met) (Amersham International plc, Buckinghamshire, UK) in 0.2 ml of saline, and killed 30 min later. Tumors were dissected and processed for microauto-radiography as described previously[2]. Silver grains were counted in various tumor regions under a microscope using a micrometer. Two to three sections of each tumor, and three tumors for each group (a total of 6 to 9 sections for each group) were analyzed for the number of grains/100 μm^2, number of grains/cell, and the number of cells/100 μm^2. Met uptake by various tumor regions was expressed as the number of grains/100 μm^2. Tumor regions were classified into four categories including AH109 A tumor cells (normal or moderately

damaged), macrophages, necrosis with scarce macrophage contamination, and young granulation tissue.

Results

Figure. 1 A illustrates Met uptake by different tumor regions in response to irradiation. Prior to irradiation, AH109 A tumor cell layer showed the highest uptake, with the uptake by granulation tissue approximately 25 % of that of tumor cells. After irradiation with 20 Gy, the uptake of Met by tumor cells decreased rapidly. In contrast, the uptake changes by granulation tissue were not significant. Macrophages and necrosis had the lowest uptake, and showed slight decreased at 2 days after irradiation.

When the uptake was expressed as the number of grains/cell, the different patterns were observed (Fig. 1B). The number of grains per tumor cell increased significantly 1 day after irradiation, then decreased on the second days. The number of grains per fibroblast in young granulation tissue increased on 2 day. The number of grains per macrophage was low.

A significant and progressive reduction in tumor cell density was observed after irradiation (Fig. 1C). Histologically, this change in tumor cell density was due to the swelling of tumor cells, representing a process of giant cell formation, an integral phenomenon of radiation injury[3]. Macrophage cell density also decreased. A rapid expansion of necrosis was observed histologically on 2 day after irradiation.

Discussion

Previous studies measured tumor uptake of tracer compounds as radioactivity within the whole tumor, expressed by the differential uptake ratio calibrated using tumor weight, injection dose, and weight of animal[1,4]. Results of the present study indicate that Met uptake by macrophages, granulation tissue, and necrotic tissue was low and showed little or no change following irradiation. On the other hand, the majority of Met uptake was due to tumor cells, decreasing sharply and rapidly following irradiation. The Met uptake by tumor cells is similar to the response by whole tumor[1,4].

Swelling of tumor cells following irradiation observed in the present study is consistent with transient increase in tumor volume following 20 Gy or 10 Gy irradiation observed in the same tumor model[1,4]. Our autoradiographic technique demonstrated an increase in Met uptake by giant cell on 1 day after irradiation. However, the decrease in cell density per unit area was large enough to reduce the net Met uptake per unit area. The latter is the only indicator measurable by PET, and its decrease after irradiation reflects metabolic degradation. *In vitro* increase in Met uptake by giant cell following irradiation was also reported by Higashi et al.[5], but this phenomenon may not fundamentally influence PET evaluation.

In conclusion, taking the PET imaging into consideration, Met uptake by tumor cell is a very important factor. The rapid response of Met uptake by tumor cell with no significant interference by non-neoplastic tissue components e. g. macrophages and granulation tissue, suggests that ^{11}C-Met is a suitable tracer for monitoring tumor radiotherapy with PET.

Acknowledgments

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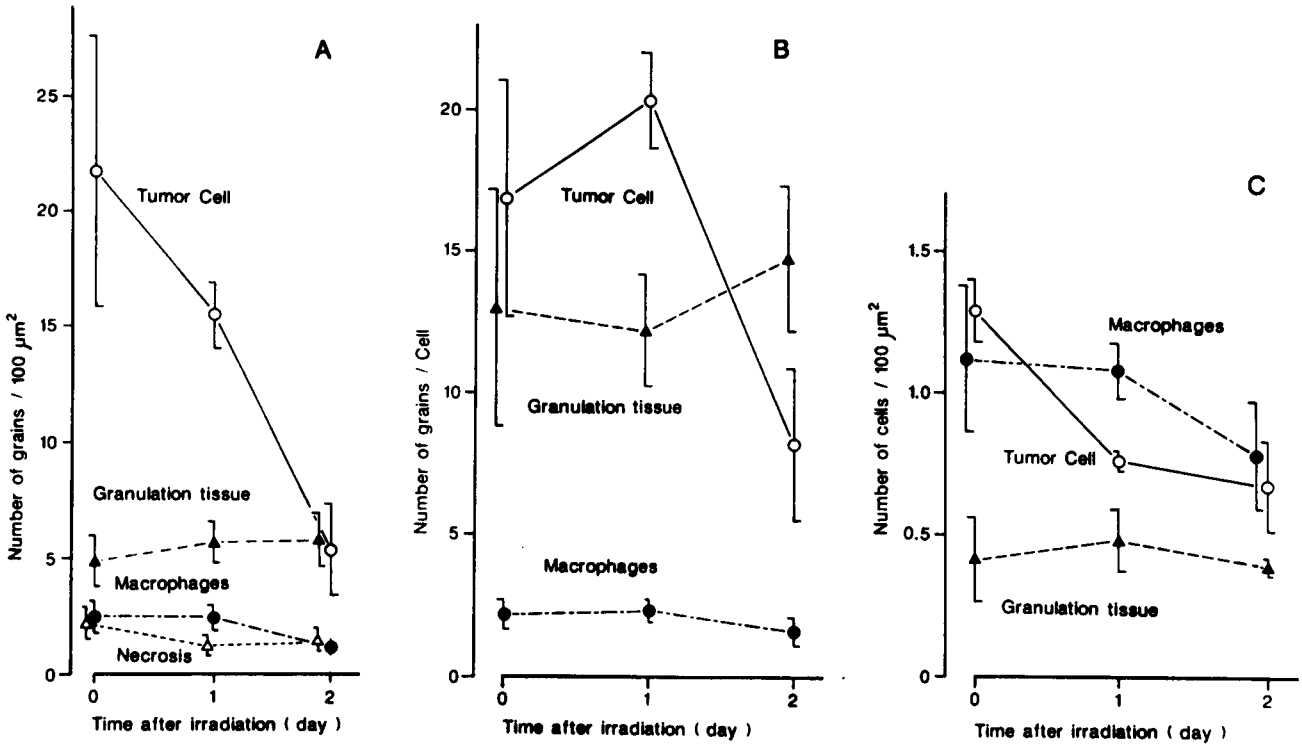

Fig. 1. Micro-autoradiographic distribution of ^{14}C-L-Methionine within AH109 A tumor after 20 Gy of irradiation. (A) ^{14}C-Met uptake was expressed as the number of silver grains per 100 μm^2. Day 0 represented control. ○: tumor cell, ▲: granulation tissue, ●: macrophages, Δ: necrosis. (B) Number of grains per cell in various histological regions. (C) Cell density of each histological regions.